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Malnutrition in African Adults

1. Serum Proteins, Cholinesterase, and Protein-bound Lipid

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The type of malnutrition most commonly seen in East Africa appears to be due to a deficiency of dietary protein. It is not 'undernutrition', i.e. deficiency of intake of calories and all dietary constituents. Nor do the symptoms suggest a deficiency of any of the known accessory food factors. That the malnutrition is due to inadequate protein intake is suggested both by a consideration of the staple foods and dietary habits of the population and also by the symptoms of adult patients admitted to hospital with a diagnosis of malnutrition.

The serum proteins are obviously of interest in such a condition because they, apart from the proteins of the liver and intestine, have the quickest rate of turnover of any body protein. The changes in serum proteins in inanition are already well known, both from the observations made during famines and food shortages (c.f. Members of the Department of Experimental Medicine, Cambridge, and Associated Workers, 1951) and from experiments made on human volunteers (Taylor, Mickelsen & Keys, 1949). However, the changes occurring in a protein deficiency without a calorie deficiency have rarely been studied, because such a state does not often occur in Europe or North America, where mixed diets are eaten. The subjects studied by Youmans, Bell, Donley & Frank (1932, 1933) may perhaps have suffered from such a condition, but it is mainly in underdeveloped parts of the world, where the diets consist of one or two foodstuffs only, that protein deficiency commonly occurs.

We were particularly interested in this question because we had already found that in apparently normal Africans, not suffering from any condition liable to cause anaemia or serum-protein changes, there is a relationship between red-cell count and serum

proteins (Holmes, Stanier, Semambo & Jones, 1951; Stanier, 1953). Those persons with the highest red-cell counts have higher albumin levels and lower globulin levels than those with lower red-cell counts; and the change in total globulin in the different red-cell-count groups occurs mainly in a fraction of the γ-globulin not precipitable with saline ammonium sulphate, as used by Wolfson, Cohn, Calvary & Ichiba (1948) for precipitating a protein fraction alleged to be γ-globulin.

Our aim was to find what characteristic changes occur in the concentration of the various serum proteins of persons suffering from protein-deficiency malnutrition, and during recovery from this condition. Since pseudo-cholinesterase has been said to be lowered in undernutrition (McCance, Widdowson & Hutchinson, 1948) as well as in liver diseases (McArdle, 1940) it was also measured in serums of our patients. In order to detect a possible qualitative abnormality, the protein-bound lipid was measured in serums of patients and normal subjects.

EXPERIMENTAL

Subjects

The patients were fourteen adult men admitted to hospital with complaints of weakness, breathlessness and palpitations, and swelling of the ankles. In the general wards of the hospital a diagnosis of malnutrition (with or without liver damage) was made. The men were then transferred to a small metabolic ward, where the diagnosis was confirmed, and were treated with a high-protein diet (approx. 180 g/day) of which reconstituted dried skim milk was the main source of protein. Most patients also received iron either by mouth or by injection. The patients were infested, more or less heavily, with hookworm; they were treated with the antihelminthic, trichloroethylene, not on admission, but at some stage during their stay in hospital.

The patients' diet before admission could not of course be known quantitatively. Inquiry revealed that they had subsisted mainly on cassava (1·5% protein) plantains (1·2% protein) and sweet potato (2% protein). Meat, fish, milk and eggs were rarely consumed by any of them. Whether or not their diets had been adequate in total calories, there had obviously been a gross imbalance between calorie intake and protein intake.

Serum proteins

Chemical analysis. (1) Total protein was measured by the micro-Kjeldahl method using copper sulphate and selenium dioxide as catalysts during incineration. The non-protein nitrogen was also measured in serums where it was expected to be raised; in other serums, the figure 23 mg/100 ml. (the mean of a large number of non-protein nitrogen estimations on African serums) was taken. (2) Albumin was measured by the method of Pillemer & Hutchinson (1945), the filtrate from the cold methanol precipitation being incinerated and distilled by the micro-Kjeldahl procedure. (3) Albumin + α-globulin was measured by the method of Wolfson *et al.* (1948), the precipitation being at about 35° and the biuret colour being read on a King photoelectric colorimeter calibrated by serum total protein. (4) γ-Globulin was measured by the method of Wolfson *et al.* (1948), the precipitation being at 20–25°. α-Globulin was given by (3)−(2), β-globulin by (1)−((3)+(4)).

Electrophoresis. The micro-electrophoresis apparatus of Antweiler was used. The serum was dialysed against barbitone buffer, pH 8·6, ionic strength 0·12. It was diluted 1 in 4 or 1 in 5 with fresh barbitone buffer before being subjected to electrophoresis. The ascending boundaries were used for measurement of total protein and of the percentage of each fraction. α_1- and α_2-globulin could be measured separately by this technique, but this was rarely done, and total α-globulin ($\alpha_1+\alpha_2$) was usually calculated. The δ artifact was rarely seen at the dilutions used. When seen it was subtracted from the total protein before calculation of the other fractions.

'*Fraction X.*' This name has been given to a component of serum proteins with mobility of γ-globulin but not precipitable with saline ammonium sulphate, and therefore measured as β-globulin by the chemical procedure. Twenty-four ml. saline ammonium sulphate, as used in the method for γ-globulin, were pipetted into a centrifuge tube and 1 ml. of serum was layered on top. After thorough mixing by inversion, the mixture was centrifuged at about 1500*g* for 30 min. The supernatant fluid was transferred quantitatively into a collodion sac and dialysed against running tap water overnight or until free of sulphate. It was then dialysed against two changes of distilled water. The precipitate ('fraction X') was centrifuged, washed twice on the centrifuge with ice-cold water, and transferred to a Kjeldahl flask for incineration. The nitrogen was measured by the micro-Kjeldahl method and multiplied by the factor 6·25 to convert to protein. The material obtained in this way shows in the Antweiler apparatus two peaks, both of low mobility. It is considered, however, to be entirely γ-globulin, not β-globulin, because it can be prepared from Cohn fraction II but not from fraction III. (Lever, Gurd, Uroma, Brown, Barnes, Schmid & Schultz (1951), who have adapted Cohn's 'Method X' to small-scale fractionation, report that fraction II contains only γ-globulins; fraction III contains β-globulins and related substances.)

Serum pseudo-cholinesterase

The method of De la Huerga, Yesinick & Popper (1952) was used. A complete set of standard solutions, containing 40, 60, 80 and 100 μmoles of acetylcholine bromide, was made for every batch of serums to be tested, and the serum samples were analysed in duplicate. The normal limits obtained by this method are 130–310 μmoles of acetylcholine hydrolysed/ml. serum/h.

Protein-bound lipid

The method of McFarlane (1942) was adapted to small-scale extractions as follows: 5 ml. serum and 1·5 ml. ether were shaken in a stoppered centrifuge tube which was then plunged into a freezing mixture (solid carbon dioxide and ethanol) contained in a Thermos jar. When the mixture was frozen solid, the tube was removed and thawed by standing in water at room temperature. The ether layer was separated by centrifugation, and the serum layer was removed from under it by a teat pipette into a clean centrifuge tube. The extraction, freezing and centrifugation were repeated twice more with 1 ml. portions of ether.

RESULTS

The values (by the chemical method) of albumin and total globulin in the patients' serums on admission to the metabolic ward are shown in Table 1. It is seen that in every case, whether or not there was clinical evidence of liver damage, the albumin was rather low and the globulin high.

Table 1. *Serum-protein levels (measured chemically) of African adults on admission to hospital*

With liver damage			Without liver damage		
Patient no.	Albumin (g/100 ml.)	Globulin (g/100 ml.)	Patient no.	Albumin (g/100 ml.)	Globulin (g/100 ml.)
1	1·69	6·90	2	3·00	4·65
5	2·25	4·85	3	1·95	3·45
8	1·04	3·95	4	2·28	3·93
11	0·63	6·60	6	0·97	4·51
13	1·06	4·93	7	1·82	3·46
14	1·11	4·17	9	1·02	5·77
	Mean 1·30	Mean 5·20	10	0·76	6·13
			12	1·70	4·03
				Mean 1·70	Mean 4·49

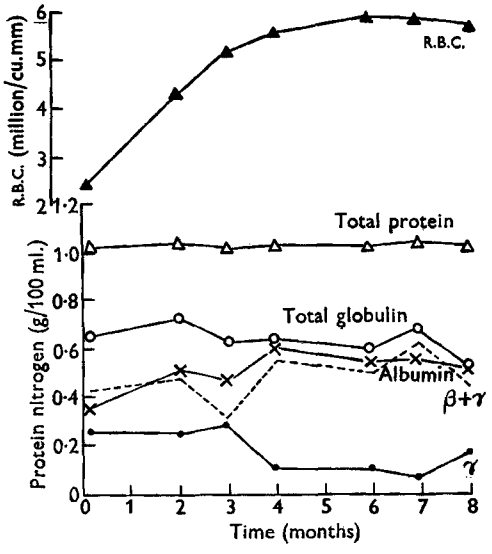

Fig. 1. Patient no. 4. Changes in red-cell count and serum proteins during treatment.

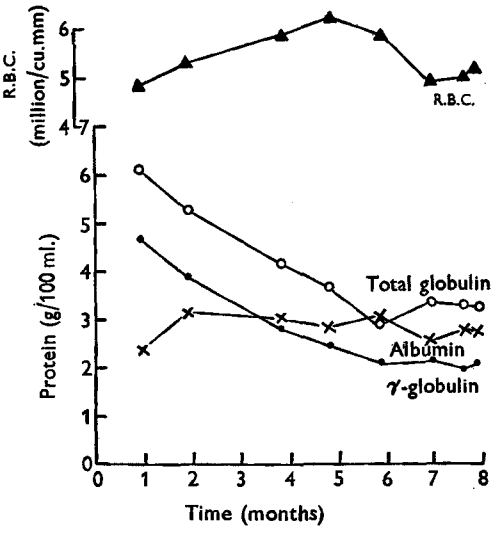

Fig. 2. Patient no. 8. Changes in red-cell count and serum proteins during treatment.

The changes occurring during recovery are shown, for two typical instances, in Figs. 1 and 2. The most striking change is the rise in the red-cell count. The albumin also rises during recovery but does not exactly parallel the red-cell count. It was expected that the globulin would fall during recovery, at least to the level found in healthy African students, i.e. about 3·3 g/100 ml. A fall occurred sometimes, but not invariably, and in five of the patients the globulin level was almost the same on admis-

sion and on discharge, though it fluctuated during treatment. In three patients there was an initial rise in globulin; all these three were admitted with very low levels of total protein.

Fig. 3 shows the results for a patient who on admission to the metabolic ward could not be persuaded to take protein as dried milk. His protein intake remained low, but he was given iron by mouth. It will be seen that, although the red-cell count rose, both albumin and globulin fell; during this time his oedema worsened. It seemed probable that haemoglobin was being made at the expense of the serum proteins. Later, the patient was induced to eat protein, and both serum proteins and red-cell count

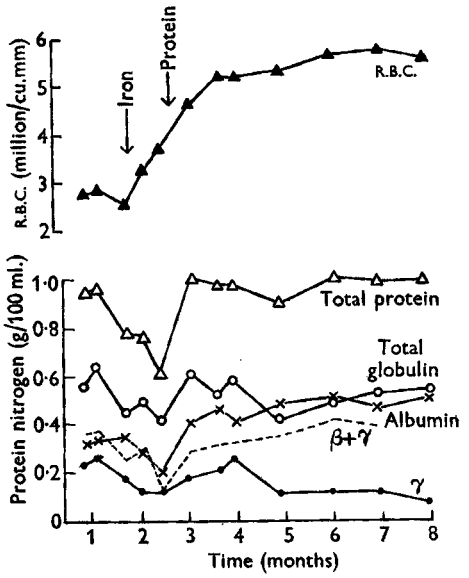

Fig. 3. Patient no. 3. Changes in red-cell count and serum proteins during treatment.

Table 2. *'Fraction X' in serums from healthy and malnourished subjects*

	'Fraction X' (mg/100 ml.)	
Subjects	Mean	Range
Five healthy Europeans	120	26–245
Four healthy Africans	325	129–445
Nine patients	475	245–675

increased. This result was of some interest as it accorded with the finding of Whipple (1942) on dogs, who, when both anaemic and protein-deficient, showed a preference for haemoglobin manufacture over plasma-protein manufacture.

The main difference between results obtained by the chemical method (of which Figs. 1 and 3 are examples) and those obtained by electrophoresis (e.g. Fig. 2) is that by the latter method the changes in total globulin are almost entirely in the γ-globulin fraction (which is seen in the graph to run parallel with total globulin), whereas by the chemical procedure the fluctuations of 'β-+γ-globulin' run parallel with that of the total globulin, but γ-globulin does not. It follows that the main change occurs in a fraction measured as β-globulin by the chemical procedure and as γ-globulin by

electrophoresis. That this component ('fraction X') is found in higher concentration in patients with malnutrition is shown by Table 2.

The fact that in groups of apparently normal Africans the albumin level rises and the globulin level falls with increasing red-cell count suggested that the same relation might obtain in a single individual as his red-cell count gradually rose during recovery from malnutrition. To find whether this was true the levels of serum albumin found in the patients at various times were plotted against the red-cell count obtained on the

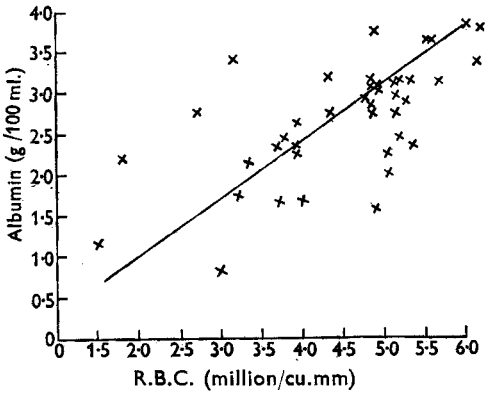

Fig. 4. Relation of red-cell count to serum albumin during recovery from protein-deficiency malnutrition. Forty-one serums from eight patients.

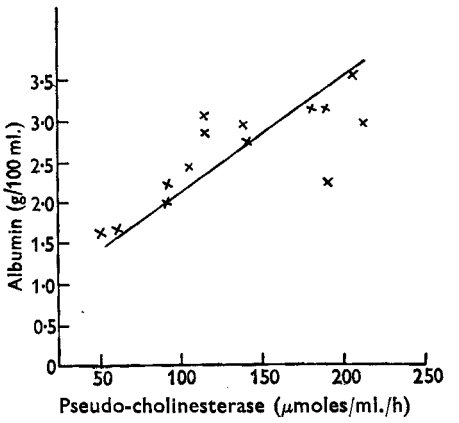

Fig. 5. Relation of serum albumin to pseudo-cholinesterase during recovery from protein-deficiency malnutrition. Fourteen serums from six patients.

Table 3. *Serum pseudo-cholinesterase and albumin in patients recovering from protein-deficiency malnutrition*

Patient no.	Date	Pseudo-cholinesterase (μmoles/ml./h)	Albumin (g/100 ml.)
7	27. v. 53	92	2·00
	30. vi. 53	105	2·45
	24. vii. 53	142	2·75
8	30. vi. 53	180	3·14
	21. vii. 53	187	3·12
11	27. vi. 53	115	2·87
	24. vii. 53	115	3·07
12	22. iv. 53	203	2·55
	26. vi. 53	138	2·92
	27. vii. 53	212	2.93
13	27. vi. 53	62	1·69
	24. vii. 53	190	2·27
14	26. vi. 53	52	1·66
	24. vii. 53	87	2·22

same day. Fig. 4 shows the result. The correlation coefficient, r, was calculated from the results, and found to be $+0{\cdot}72$ ($P<0{\cdot}001$), showing a high degree of correlation. The procedure was repeated for γ-globulin with red-cell counts, but no correlation was obtained ($r=-0{\cdot}33$).

Serum pseudo-cholinesterase was measured at intervals in a number of the patients. The results are shown in Table 3, together with the value of serum albumin found on

the same day. It will be seen that in most cases the cholinesterase was low initially and rose during treatment, and that the cholinesterase tended to run parallel with the albumin. (Patients nos. 8 and 12 had been under treatment for some months before the cholinesterase was measured.)

The correlation between pseudo-cholinesterase and albumin levels is obvious if the results are plotted on a graph (Fig. 5). The correlation coefficient, r, is $+0{\cdot}74$ ($P<0{\cdot}001$), showing a significant correlation. No correlation was found, however, between cholinesterase and albumin levels in normal persons. This finding confirms the observations of Levine & Hoyt (1950).

It was possible that there was a qualitative as well as a quantitative difference from normal in the serum proteins of patients with malnutrition. For instance, the distribution of lipid between the various fractions might be changed. To test this possibility, a number of serums were extracted with ether at $-60°$ to remove the lipid bound to protein, and the electrophoresis patterns before and after extraction were compared. The results were calculated on the assumption that lipid causes the same phase difference in the Antweiler apparatus as the corresponding weight of protein. As a preliminary, it was found that no appreciable change was made in the pattern by shaking the serum with ether and standing the mixture overnight at ice-box temperature. There was therefore no easily removable lipid, such as that in the serums of persons with lipid nephrosis.

The results of the frozen ether extractions are shown in Table 4. The results on eight serums from five normal Europeans and one healthy African student are compared with the results on seven serums from seven patients. The patients were on the generous diet of the research ward, though two of them had been taking this diet for only about 10 days before their serums were studied; the other patients had been eating this diet for weeks or months. It contained 3000 Cal. or more daily, the fat intake being 30–60 g/day.

The decrease in (apparent) total protein after lipid extraction was clearly much greater in the normal persons. The changes in albumin and γ-globulin levels were variable and are not recorded in the table; sometimes there was a slight decrease and sometimes apparently a slight increase, but the change was rarely as much as 10% of the original level, in either normal persons or patients. This result was expected, as albumin and γ-globulin are not known to carry lipid. The changes in α- and β-globulin levels, however, are obvious. There was a pronounced drop in almost every instance. β_2-Globulin and α_1-globulin are known to carry lipid. (In a few cases—two normals and one patient—where the α_1- and α_2-globulins were measured separately, the α_1-globulin fell greatly, while the α_2 fraction fell slightly or was unchanged.)

For α-globulin the mean initial levels of the normal persons and patients were 0·95 and 0·64 g/100 ml. respectively, a barely significant difference ($t=2{\cdot}26$, $P=0{\cdot}05-0{\cdot}1$). For β-globulin, the mean initial levels were 0·68 and 0·69 g/100 ml. clearly not significantly different. A difference in concentration of the lipid-bearing proteins cannot therefore account for the patients' very much lower level of protein-bound lipid. There must have been a smaller percentage of lipid in the lipid-bearing globulins of patients than in those of normal persons.

Table 4. *Changes in serum protein after lipid removal of eight serums from five normal Europeans and one healthy African student and of seven serums from seven patients*

Change in 'total protein'				Change in 'β-globulin'				Change in 'α-globulin'			
Normal subjects		Patients		Normal subjects		Patients		Normal subjects		Patients	
g/100 ml.	Percentage of initial	g/100 ml.	Percentage of initial	g/100 ml.	Percentage of initial	g/100 ml.	Percentage of initial	g/100 ml.	Percentage of initial	g/100 ml.	Percentage of initial
−0·87	−13·4	0	0	−0·27	−53	−0·13	−19	−0·56	−45	−0·23	−38
−0·71	−11·2	−0·26	−4·1	−0·16	−53	−0·41	−36	−0·62	−44	−0·09	−12
−0·87	−13·5	−0·18	−2·8	−0·40	−55	−0·22	−31	−0·53	−44	−0·07	−9
−0·85	−12·5	−0·23	−3·2	−0·66	−67	−0·23	−36	−0·28	−29	0	0
−1·50	−25·0	−0·25	−4·8	—	—	—	—	—	—	—	—
−1·24	−16·6	−0·28	−4·1	−0·16	−55	−0·28	−48	−0·40	−65	−0·02	−3
−0·49	−7·0	−0·15	−2·5	−0·34	−41	−0·03	−7	−0·78	−64	0	0
−1·62	−22·4	—	—	−0·36	−55	−0·35	−51	−0·15	−25	−0·08	−21
—	—	—	—	−0·27	−36	—	—	−0·01	2	—	—
Mean											
−1·02	−15·2	−0·19	−3·1	−0·34	−49	−0·24	−33	−0·42	−40	−0·07	−12

DISCUSSION

The origin of the high total globulin and γ-globulin in our subjects calls for explanation. One might have expected that on a low protein intake all the blood proteins would decrease. The patients of Youmans *et al.* (1932, 1933) showed an initial great increase in globulin at the beginning of treatment, followed by a slower increase in albumin; then the globulin fell while the albumin continued to rise. The nearest parallel with our observations is found in the patients (victims of concentration camps) studied by Gsell (1945). These persons all had a low albumin and a marked increase in globulin, mainly in the γ-globulin fraction. However, these subjects had certainly been suffering from a deficiency of total calories as well as of protein. It is possible that the explanation of the raised γ-globulin is the same as that put forward to account for the γ-globulin of cirrhosis (Szanto & Popper 1951): an increased protein synthesis by the macrophages infiltrating the portal triads of the damaged liver. Davies (personal communication) has found macrophage infiltration in portal triads of the great majority of Africans seen by him at post-mortem; but it is not known whether these macrophages show signs of increased protein synthesis.

It is interesting that the rise in serum pseudo-cholinesterase should run parallel with that of albumin. A correlation between albumin and pseudo-cholinesterase has been found in a number of conditions in which pseudo-cholinesterase is low (Alcalde, 1950; Levine & Hoyt, 1950). The most probable explanation is on the lines suggested by Levine & Suran (1952) that pseudo-cholinesterase reflects the ability of the liver cells to synthesize protein. Hutchinson, McCance & Widdowson (1951) reported no correlation between pseudo-cholinesterase and serum albumin or total protein in their subjects recovering from undernutrition. It is possible that this correlation is present only in a specific protein deficiency, not in a generalized undernutrition. However, an examination of the data of Hutchinson *et al.* (1951) reveals that the subjects whose diet during refeeding was supplemented with protein-rich foods (or fats) increased their pseudo-cholinesterase far more rapidly than those who were given carbohydrate supplements. It would have been interesting to try to detect changes in serum albumin in these groups, but this is not reported.

The only qualitative difference in serum proteins between patients with malnutrition and normal subjects so far detected is the much smaller percentage of lipid bound to lipoproteins (α- and β-globulins) in the patients. Both these globulins are thought to carry steroids, as well as phospholipid (Marrack & Hoch, 1949). Unpublished observations by Davies, and by Holmes in this laboratory, suggest that in severe malnutrition as seen in Uganda there is atrophy of adrenal cortex (detected in sections) and abnormal cortical function (shown by lack of reponse to ACTH). If these observations are confirmed, they may help to explain the comparatively small amount of protein-bound lipid in such patients.

SUMMARY

1. Adult men suffering from protein-deficiency malnutrition were treated with a high-calorie high-protein diet. During treatment their red-cell count, serum proteins, serum pseudo-cholinesterase and protein-bound lipid were studied.

2. At the beginning of treatment, whether or not the patients had clinical evidence of liver damage, they had low levels of serum albumin and raised levels of globulin. The 'fraction X', a component with the mobility of γ-globulin but estimated chemically as β-globulin, was on an average higher in patients than in normal Africans or Europeans.

3. During treatment with a high-calorie, high-protein diet, the serum albumin (and red-cell count) rose. The changes in globulin were not consistent, but the fluctuations occurred mainly in the component measured as γ-globulin by electrophoresis and as β-globulin by the chemical method.

4. Serum pseudo-cholinesterase was low in such patients and rose as the serum albumin increased.

5. There was less lipid on the lipo-proteins in persons with malnutrition than in normal subjects.

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